of BC present in equine blood for use in diagnosis of equine babesiasis. Such a protein and a polypeptide constituting a portion of said protein may also be used as an antigen for preparing anti-BC merozoite antibodies, especially a monoclonal antibody to BC merozoite. The anti-BC merozoite antibody thus prepared may be used for detecting BC merozoite in equine blood for use in diagnosis of equine babesiasis.

EXAMPLE

The present invention is explained in more detail by means of the following Examples but it should not be construed to be limited thereto.

Example 1: Construction of cDNA Library of *Babesia caballi* Merozoite

BC-infected erythrocytes with about 10% of a rate of parasite within erythrocytes were prepared by *in vitro* culture as described by Avarzed et al. [J. Vet. Med. Sci. 59(6), 479-481 (1997)]. That is, blood was drawn from horses infected with BC (USDA strain) into a tube charged with EDTA as a coagulating agent. The tube was centrifuged with RPMI1640 medium supplemented with 10 mM HEPES and washed and buffy coat was removed. After centrifugation and washing, a supernatant was discarded and sediment 50 µl was mixed with 1 ml of RPMI1640 medium (containing 2 mM L-glutamine and 50 µl normal equine erythrocyte) supplemented

with 40% equine serum. The mixture was added to a 24-well microtiter plate at 1 ml/well. The microtiter plate was incubated at 37°C with conditions of 5% CO_2, 2% O_2 and 93% N_2. While incubation, the culture medium was replaced with fresh medium and a rate of parasite was measured by the Giemsa staining everyday with passage being performed whenever appropriate.

From the thus obtained BC-infected erythrocytes, cDNA library was constructed as reported by Ikadai et al. [The 126th Japan Veterinary Association, excerpt, page 191 (1998)]. That is, total RNAs were extracted from the BC-infected erythrocytes by the guanidinium-phenol-chloroform method as described by Chomczynski et al. [Anal. Biochem. 162, 156-159 (1987)]. mRNAs were isolated and purified from the total RNAs with oligotex-dT 30 (manufactured by Takara K.K.) and then cDNAs were synthesized with Zap-cDNA synthesizer kit (manufactured by Stratagene Inc.) in accordance with the protocol attached thereto. The cDNAs were inserted into λZap II phage vector (manufactured by Stratagene Inc.) and packaged with Gigapack III packaging system (manufactured by Stratagene Inc.) in accordance with the protocol attached thereto to construct cDNA library.

Example 2: Production of Monoclonal Antibody Recognizing 48kDa Antigen of *Babesia caballi* Merozoite

As an antigen, a suspension of 1×10^8 merozoite

from BC-infected horses in 0.1 ml phosphate buffer was emulsified with Freund's complete adjuvant (manufactured by Difco). The emulsion (0.2 ml/mouse) was inoculated intraperitoneally and subcutaneously to BALB/c mice of 7 weeks old. A suspension of the same amount of merozoite with Freund's incomplete adjuvant (manufactured by Difco) was boostered three times with intervals of two weeks. Three days after the fourth immunization, merozoite was administered intravenously to mice. Three days later mice were dissected and the spleen was removed. The spleen cells were fused with Sp-2 mouse myeloma cells with polyethylene glycol (PEG 1500, manufactured by Boehringer Mannheim Biochemica).

The hybridoma cells were selected with HAT medium (manufactured by Boehringer Mannheim Biochemica) and GIT medium (manufactured by Wako K.K.) supplemented with Bri Clone (manufactured by BioResearch) in conventional manner. The hybridoma cells were screened for their supernatant by the indirect fluorescent antibody procedure with smear of BC-infected erythrocytes fixed with cold acetone to thereby give six clones. Among these, monoclonal antibodies produced by two hybridomas, referred to as "BC11D" and "BC233", were found to recognize the same 48kDa antigen by Western blot with solubilized antigen of BC merozoite. It was confirmed that neither of the monoclonal antibodies